The vitamin A and vitamin E status of horses raised in Alberta and Saskatchewan

Barry R. Blakley, Roxy J. Bell

Abstract

The purpose of the study was to determine normal baseline levels of vitamin A and vitamin E in clinically normal horses under typical field conditions in Saskatchewan and Alberta. Heparinized blood samples were collected from approximately 400 clinically healthy horses selected from 24 locations in Alberta and Saskatchewan during a two-year period. For each horse, historical information including feed type, vitamin supplementation, time of year, sex, and age were recorded. From each blood sample, the plasma vitamin A (all-transretinol) and vitamin E (α-tocopherol) levels were measured using high pressure liquid chromatography. Normal baseline plasma vitamin A and vitamin E concentrations recorded during the study were 0.70 μmol/L and 7.65 μmol/L, respectively. The plasma vitamin concentrations were lower in the younger horses. The plasma vitamin levels were higher from May to August, as compared to other times of the year. Horses grazing fresh pasture exclusively during the summer months had plasma vitamin A and vitamin E concentrations that were 27% and 63% greater than horses fed harvested or stored feeds during the same time period. Sex-related differences were not evident in the study. A number of factors may influence the baseline plasma vitamin A and vitamin E levels in horses. Consequently, it is unadvisable to use a single evaluation to assess vitamin status. Multiple sampling from individual horses or sampling from many horses within a herd may reduce the variability and improve the ability to monitor vitamin status from plasma submissions.

Résumé

Le taux de vitamines A et E chez les équins de l'Alberta et de la Saskatchewan

Cette étude avait pour but de déterminer les valeurs de base normales pour les vitamines A et E chez les chevaux gardés au pâturage. Des échantillons sanguins héparinisés ont été prélevés chez 400 chevaux en santé répartis dans 24 endroits en Alberta et en Saskatchewan sur une période de deux ans. Le taux plasmatique des vitamines A (all trans-rétinol) et E (α-tocopherol) a été mesuré par épreuve chromatographique. De plus, les données suivantes ont été notées pour chaque animal: la sorte de nourriture, les suppléments de vitamines, la période de l'année, le sexe et l'âge. Les valeurs plasmatiques normales pour les vitamines A et E ont été respectivement 0.70 umol/L et 7.65 umol/L.

Les résultats montrent un taux plasmatique en vitamines plus faible chez les jeunes chevaux et des valeurs plus élevées pour la période de mai à août. Les chevaux nourris exclusivement à l'herbe fraîche du pâturage lors des mois d'été avaient une concentration plasmatique plus élevée en vitamines A et E, soit de 27 % et 63 % comparativement aux chevaux nourris avec du foin coupé ou entreposé. Il n'y avait pas de différences significatives reliées au sexe. L'étude démontre que plusieurs facteurs peuvent influencer les valeurs plasmatiques de base des vitamines A et E chez les équins. Alors, il est recommandé de prélever plusieurs échantillons d'un même individu ou d'effectuer un échantillonnage sur plusieurs chevaux d'un troupeau afin de diminuer la variabilité et d'améliorer le suivi du statut vitaminique plasmatique. *(Traduit par Dr Thérèse Lanthier)*

Can Vet J 1994; 297–300

Introduction

Vitamin nutritional requirements have been poorly defined for horses compared to other livestock species. Vitamin supplementation of rations is commonplace in western Canada, since the vitamin content of many stored feeds or pelleted rations is marginal or inadequate for proper growth and development in horses. In many instances, veterinarians and nutritionists may not have sufficient information to evaluate or develop optimal rations. Optimal amounts of vitamins in rations may be influenced by many factors. Rations formulated for pleasure horses or performance horses may be substantially different with respect to vitamins. Other factors including breed, sex, season, age, pregnancy, and disease may alter vitamin requirements (1–5). The long winters in western Canada that dictate the consumption of stored feeds for extended periods of time may further complicate vitamin A and E nutrition, because the stability and retention of adequate levels of these vitamins in stored feed is extremely poor (6).

Two liposoluble vitamins of particular importance in the horse are vitamin A and vitamin E. Vitamin A (retinol) has been associated with a variety of disease conditions (2). Vitamin A is required for vision, development of epithelial cells and bone, and for reproduction (6,7). Reduced resistance to disease, congenital defects, nerve degeneration, infertility, weight loss, night blindness and hoof defects may also be observed with vitamin A deficiency (2). Vitamin E (α-tocopherol) is an antioxidant, which is required to prevent lipid peroxidation and membrane damage. In horses, nutritional

Department of Veterinary Physiological Sciences (Blakley), Department of Herd Medicine and Theriogenology (Bell), Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0.

This research was supported by a grant from the Alberta Agricultural Research Institute, Farming for the Future Research Program.

Table 1. The effect of age[a] on the plasma vitamin A and vitamin E concentrations[b] of horses raised in Saskatchewan and Alberta

Age (months)	Vitamin A concentration (μmol/L)	Vitamin E concentration (μmol/L)
< 6	0.57 ± 0.04 (18)[c]	8.60 ± 1.19 (19)
6–12	0.55 ± 0.01 (57)	5.62 ± 0.52 (61)
> 12–24	0.63 ± 0.02 (35)	6.24 ± 0.30 (33)
> 24–72	0.68 ± 0.02 (91)	7.58 ± 0.30 (96)
> 72	0.70 ± 0.02 (140)	8.79 ± 0.28 (138)
Probability[d]	< 0.0001	< 0.0001

[a] Across all categories pertaining to sex, feed type, and season
[b] Vitamin A — all-trans retinol; vitamin E — α-tocopherol
[c] Mean ± SE (number of horses)
[d] Probability of no age-related effect

Table 2. The effect of sex differences[a] on the plasma vitamin A and vitamin E concentration[b] of horses raised in Saskatchewan and Alberta

Sex	Vitamin A concentration (μmol/L)	Vitamin E concentration (μmol/L)
Female	0.68 ± 0.02 (196)[c]	8.20 ± 0.25 (200)
Female (neutered)	0.60 (1)	6.15 (1)
Male	0.59 ± 0.02 (46)	6.62 ± 0.57 (45)
Male (neutered)	0.66 ± 0.01 (123)	7.47 ± 0.27 (125)
Probability[d]	0.0861	0.0564

[a] Across all categories pertaining to age, feed type, and season
[b] Vitamin A — all-trans retinol; vitamin E — α-tocopherol
[c] Mean ± SE (number of horses)
[d] Probability of no sex-related effect

muscular dystrophy is a vitamin E/selenium responsive condition, manifested by weakness, trembling, a stiff gait, and recumbency (2). Vitamin E deficiency will also impair immune function in horses (8).

The purpose of the present study was to determine the normal baseline levels of vitamin A and vitamin E in clinically normal horses under typical field conditions in Saskatchewan and Alberta, and to identify environmental and biological factors that may influence plasma vitamin concentrations.

Materials and methods

Animal population and sample collection

Clinically healthy horses, based upon visual inspection, were selected for the study from 24 locations in Alberta and Saskatchewan over a two-year period. Heparinized blood samples were collected from 10 to 20 horses at each location. The plasma was removed and frozen at −70°C until vitamin analysis could be completed. For each horse, information including pasture or stored feed, vitamin supplementation, time of year, sex, and age was recorded.

Vitamin analysis

Plasma vitamin A (all-trans retinol) and vitamin E (α-tocopherol) levels were measured using high pressure liquid chromatography (HPLC) with ultraviolet detection at 325 nm and 285 nm, respectively (9,10). Working standards of α-tocopherol, α-tocopheryl acetate, all-trans retinol (retinol), and all-trans retinol acetate (retinol acetate) were prepared in glass distilled absolute ethanol (All standards: Eastman Kodak, Rochester, New York, USA). All working standards were protected from the light and stored at −20°C prior to use.

Two milliliter plasma samples were mixed with 0.4 mL of the internal standard (retinol acetate or α-tocopheryl acetate) and 1.6 mL of absolute ethanol. The standard curve was prepared using 2.0 mL of a 3% bovine serum albumin solution (Sigma Chemical Co., St. Louis, Missouri, USA) to approximate 2.0 mL of plasma. A retinol or α-tocopherol working standard was added to each albumin solution to prepare the standard curve.

The 4.0 mL sample preparations were vortically mixed with 4.0 mL of petroleum ether (Petroleum Spirit Omni Solv; b.p. 30−60°C; BDH, Edmonton, Alberta). Three-fourths of the petroleum ether phase were removed and evaporated under nitrogen and darkness. The vitamin residue was dissolved in filtered HPLC grade methanol (BDH) and injected onto an HPLC column (Beckman ODS-ultrasphere 5 μm column, 4.6 × 15.0 cm, Beckman Instruments, Inc., Fullerton, California, USA). A degassed, filtered, methanol:water mobile phase at a v/v ratio of 90:10 for vitamin A and

Table 3. The effect of season[a] on the plasma vitamin A and vitamin E concentration[b] of horses raised in Saskatchewan and Alberta

Season	Vitamin A concentration (μmol/L)	Vitamin E concentration (μmol/L)
January–April	0.64 ± 0.01 (222)[c]	7.00 ± 0.17 (221)
May–August	0.76 ± 0.04 (89)	9.68 ± 0.44 (92)
September–December	0.53 ± 0.03 (68)	6.98 ± 0.46 (72)
Probability[d]	< 0.0001	< 0.0001

[a] Across all categories pertaining to age, sex, and feed type
[b] Vitamin A — all-trans retinol; vitamin E — α-tocopherol
[c] Mean ± SE (number of horses)
[d] Probability of no seasonal effect

97:3 for vitamin E was used to elute the respective vitamins.

Each sample was injected onto the column with an injector system (Rheodyne #7161, Rheodyne, Cotati, California, USA) equipped with a 50 μL loop. The flow rate (2.5 mL/min) and the pressure of the isocratic solvent system were regulated using an automated gradient controller (Water 680, Millipore Waters, Chromatography Division, Milford, Massachusetts, USA) and an HPLC pump (Water 501, Millipore Waters). The absorbances of vitamin A, monitored at 325 nm, and vitamin E, monitored at 285 nm, were measured using a tunable absorbance detector (Waters 484, Millipore Waters) and recorded using a data module (Water 745, Millipore Waters).

Under the previously stated analytical conditions, the retention times of retinol and retinol acetate were approximately 3.4 and 6.6 min, respectively. The detection limit for all-trans retinol was 0.04 μmol/L in plasma. The corresponding retention times for α-tocopherol and α-tocopheryl acetate were 5.5 and 8.5 min respectively. The detection limit for α-tocopherol was 0.5 μmol/L.

Data and analysis

The effects of age, sex, feed type, and season on the normal baseline values of vitamin A and vitamin E were determined using a one-way analysis of variance. Separate analyses were conducted for each factor using the total information that was collected. In each instance, the information was categorized into groups for analysis according to the number of possible group classifications.

Results

Based upon information collected from almost 400 horses over a two-year period, the normal plasma vitamin A concentration was 0.70 μmol/L. The corresponding value for the vitamin E concentration was 7.65 μmol/L. Table 1 indicates that age influenced the plasma vitamin A and vitamin E concentrations. Younger horses have a lower vitamin A and vitamin E status. Table 2 indicates that sex of the horse did not influence the vitamin status.

The highest plasma vitamin E and vitamin A concentrations were recorded from May to August (Table 3). This seasonal effect was consistent with the type of feed that is consumed at different times of the year. Horses raised exclusively on fresh pasture feeds had higher plasma vitamin concentrations. The plasma vitamin A levels were 27% higher in horses consuming only pasture feeds. In 64 horses raised on pasture, the average plasma vitamin A concentration was 0.85 ± 0.05 μmol/L as compared to 0.67 ± 0.01 μmol/L in 315 horses consuming harvested feeds ($p = 0.0001$). The plasma vitamin E levels were 63% higher in horses raised on pasture than horses fed harvested, dried, or pelleted rations. The respective concentrations for 60 horses raised on pasture were 11.35 ± 0.49 μmol/L as compared to 6.96 ± 0.16 μmol/L in 323 horses fed harvested feeds ($p = 0.0001$).

Discussion

The normal plasma vitamin A and vitamin E concentrations observed in this study compare favourably with other reports (1,3–5,11–15), although specific geographical, environmental, or physiological differences are apparent.

The present study indicated that older horses had a higher plasma concentrations of vitamins A and E. During periods of rapid growth, the nutritional demand for vitamins is high, so it is not surprising that young, rapidly-growing horses have a lower vitamin status than older horses. The vitamin E status of horses less than six months of age appears to be an exception. It has been suggested that young horses aged four to six months have a higher vitamin E status because of the ingestion of pasture feeds high in vitamin E (4). It may also be possible that fat soluble vitamins, such as vitamin E, may be passed in the milk or colostrum of the mare to the offspring, which could elevate the vitamin E concentrations in the plasma for several months. This latter effect has been reported for vitamin A (14), although similar observations have not been confirmed with respect to vitamin E. Age-related effects associated with vitamin nutrition have been documented to a limited extent by others (1,4).

Probably the most significant factor influencing the vitamin status in horses identified in this study was the seasonal variability, which is, in part, attributed to the type of feeds consumed by horses at different times of the year. The highest plasma vitamin A and vitamin E levels were recorded during the period from May to August. For the most part, this is the only time of the year that horses in western Canada can consume feed containing high levels of naturally-occurring vitamins. Horses grazing pasture exclusively during this period had substantially higher levels of plasma vitamin A or vitamin E. Both vitamin A and vitamin E have limited stability in feeds. The vitamins may degrade easily or become oxidized upon long-term storage, heating, grinding, or pelleting of feed (6). It has been reported (1) that processed feeds may not provide adequate levels of vitamins in pregnant and weanling horses during the winter months. Seasonal variability associated with vitamin A and vitamin E have been reported by others (1,3,5). In addition to the seasonal differences, considerable variability in the plasma or serum vitamin levels may be observed during a short period of time. In one study (16), the serum vitamin E concentrations fluctuated dramatically over a 72 hour period. The serum vitamin E/cholesterol ratio was also determined to be a poor indicator of vitamin E status. It was suggested that multiple sampling from the same horse may provide a more reliable indicator of vitamin E status. If multiple sample collection is not feasible, individual blood samples from a group of horses with a similar nutritional background and comparable biological characteristics may provide a more informative estimate of the vitamin status.

It is evident from the present study that a lower vitamin status may be associated with winter feeding of harvested and stored feeds or young rapidly growing horses. Horses that fall into one or more of these categories are more likely to have a low vitamin status. Vitamin supplementation under these circumstances is desirable. In contrast, supplementation is probably not required for horses that are consuming fresh feed or grazing on pasture. When horse rations are formulated for optimal growth and development, factors influencing vitamin requirements and availability identified in this study need to be considered. To evaluate the existing vitamin status, one blood sample from an individual is insufficient to reliably estimate the vitamin status. CVJ

Acknowledgments

The authors thank Mrs. L. Kumor and Mrs. A. Nowak for their excellent technical assistance during this project.

References

1. Maenpaa PH, Koskinen T, Koskinen E. Serum profiles of Vitamins, A, E and D in mares and foals during different seasons. J Anim Sci 1988; 66: 1418–1423.
2. Blood DC, Radostits OM. Veterinary Medicine, 7th ed. London: Baillière Tindall, 1989: 1187–1202, 1218–1223.
3. Maenpaa PH, Lappetelainen R, Virkkunen J. Serum retinol, 24-hydroxy vitamin D and α-tocopherol of racing trotters in Finland. Equine Vet J 1987; 19: 237–240.
4. Blythe LL, Craig AM, Lassen ED, Rowe KE, Apell LH. Serially determined plasma α-tocopherol concentrations and results of the oral vitamin E absorption test in clinically normal horses and in horses with degenerative myeoloencephalopathy. Am J Vet Res 1991; 52: 908–911.
5. Maylin GA, Rubin DS, Lein DH. Selenium and vitamin E in horses. Cornell Vet 1980; 70: 272–289.
6. Schryver HF, Hintz HF. Vitamins. In: Robinson EN, ed. Current Therapy in Equine Medicine, 2. Philadelphia: WB Saunders Company, 1987: 405–409.
7. Putnam ME. The role of vitamins in nutrition of performance horses. Mod Vet Pract 1986; 67: 121–124.
8. Baalsrud KJ, Overnes G. Influence of vitamin E and selenium supplement on antibody production in horses. Equine Vet J 1986; 18: 472–474.
9. Milne DB, Botmen J. Retinol, α-tocopherol, lycopene and α- and β-carotene simultaneously determined in plasma by isocratic liquid chromatography. Clin Chem 1986; 32: 874–876.
10. Catignani GL, Biere JG. Simultaneous determination of retinol and alpha-tocopherol in serum or plasma by liquid chromatography. Clin Chem 1983; 29: 708–712.
11. Baker H, Schor SM, Murphy BD, DeAngelis B, Feingold S, Frank O. Blood vitamin and choline concentrations in healthy domestic cats, dogs and horses. Am J Vet Res 1986; 47: 1468–1471.
12. Garton CL, Vander Noot GW, Fonnesbeck PV. Seasonal variation in carotene and vitamin A concentration of the blood of brood mares in New Jersey. J Anim Sci 1964; 23: 1233.
13. Sklan D, Donoghue S. Serum and intracellular retinol transport in the equine. Br J Nutr 1982; 47: 273–280.
14. Stowe HD. Vitamin A profiles of equine serum and milk. J Anim Sci 1982; 54: 76–81.
15. Butler P, Blackmore DJ. Vitamin E values in the plasma of stabled thoroughbred horses in training. Vet Rec 1983; 112: 60.
16. Craig AM, Blythe LL, Lassen ED, Rowe KE, Barrington R, Slizeski M. Variations of serum vitamin E, cholesterol, and total lipid concentrations in horses during a 72 hour period. Am J Vet Res 1989; 50: 1527–1531.